

CLAIMS

1. A biologically pure bacterial culture of *M. elsdenii* having substantially the same 16S ribosomal RNA sequence as that of the *M. elsdenii* strain deposited at NCIMB, Aberdeen, Scotland, UK under number NCIMB 41125.
2. A biologically pure bacterial culture of the *M. elsdenii* strain deposited at NCIMB, Aberdeen, Scotland, UK under number NCIMB 41125.
3. A biologically pure bacterial culture of *M. elsdenii* according to claim 1 or claim 2 which is further characterised by its ability to utilise lactate very efficiently even in the presence of sugars; its resistance to ionophores; its relatively high growth rate; its capability to produce predominantly acetate; and its capability to proliferate at relatively low pH values below 5.0 and as low as 4.5.
4. A composition for facilitating the adaptation of ruminants from a roughage-based diet to a high-energy concentrate-based diet, the composition consisting essentially of a bacterial culture according to any one of claims 1 to 3.

5. A method of facilitating the adaptation of ruminants from a roughage-based diet to a high-energy concentrate-based diet including the step of administering to the rumen of said ruminants an effective amount of a bacterial culture according to any one of claims 1 to 3.
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6. A feed-additive for ruminants comprising a carrier and an effective amount of a bacterial culture according to any one of claims 1 to 3.
7. A feed-additive according to claim 6 wherein the culture is disposed in an anaerobic container.
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8. A method for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic acidosis induced bloat and liver abscesses, including the step of anaerobically administering to the rumen of a ruminant an effective amount of a bacterial culture according to any one of claims 1 to 3.
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9. A veterinary agent for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic acidosis induced bloat and liver abscesses, comprising an
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effective amount of a bacterial culture according to any one of claims 1 to 3.

10. A preparation for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic acidosis induced bloat and liver abscesses in ruminants comprising an inoculum of a bacterial culture according to any one of claims 1 to 3; and a separate sterile anaerobic growth medium, the components of the preparation being disposed in separate chambers of an anaerobic container which are anaerobically connectable to each other, thus to inoculate the growth medium with the culture anaerobically.
11. A method of achieving any one or more of the following improvements in ruminants namely increased milk production; improved feedlot performance; improved growth rate; decrease in finishing time; lower digestive morbidity and mortality; lower incidence of lactic acidosis and related diseases; improved feed conversion efficiency; decrease in roughage content in feeds; and capability to feed on relatively higher concentrate diets, including the step of administering to the rumen of a ruminant an effective amount of a bacterial culture according to any one of claims 1 to 3.

12. A method according to claim 11 wherein the culture is administered anaerobically.
13. A method of isolating a biologically pure culture of a superior ruminal microorganism in a relatively shorter time period than conventional methods, the method including the steps of obtaining a sample of ruminal fluids; and cultivating the sample on a pre-selected growth medium, the method being characterised in that a plurality of parameters selected from the group comprising growth medium constituents, dilution rate, pH, temperature, anti-microbial agents, gaseous environment, redox potential, lack of nutrients and challenging organisms, are pre-selected to favour the superior rumen microorganism to the detriment of inferior rumen microorganisms.
14. A biologically pure bacterial culture of *M. elsdenii* substantially as herein described and exemplified.
15. A composition for facilitating the adaptation of ruminants from a roughage-based diet to a high-energy concentrate-based diet substantially as herein described and exemplified.

16. A method of facilitating the adaptation of ruminants from a roughage-based diet to a high-energy concentrate-based diet substantially as herein described and exemplified.
- 5 17. A feed-additive for ruminants substantially as herein described and exemplified.
- 10 18. A method for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic acidosis induced bloat and liver abscesses, substantially as herein described and exemplified.
- 15 19. A veterinary agent for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic acidosis induced bloat and liver abscesses, substantially as herein described and exemplified.
- 20 20. A preparation for the treatment of ruminal lactic acidosis and prevention of any one or more of the following, namely ruminal lactic acidosis, rumenitis, ruminal lactic acidosis induced laminitis, ruminal lactic

acidosis induced bloat and liver abscesses in ruminants substantially as herein described and exemplified.

21. A method of achieving any one or more of the following improvements in
5 ruminants namely increased milk production; improved feedlot
performance; improved growth rate; decrease in finishing time; lower
digestive morbidity and mortality; lower incidence of lactic acidosis and
related diseases; improved feed conversion efficiency; decrease in
roughage content in feeds; and capability to feed on relatively higher
10 concentrate diets, substantially as herein described and exemplified.

22. A method of isolating a biologically pure culture of a superior ruminal
microorganism in a relatively shorter time period than conventional
methods, substantially as herein described and exemplified.
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REFERENCES

- 5 1. Abdo, K.M. & Cahilly, G.M. (1974). Ruminant feed additive and method of preparing the same. United States Patent **3857971**, 1-30.
2. Allison, M.J., Bucklin, J.A. & Dougherty, R.W. (1964). Ruminant changes after overfeeding with wheat and the effect of intraruminal inoculation on adaptation to a ration containing wheat. *J Anim Sci* **23**, 1164-1170.
- 10 3. Braun, U., Rihs, T. & Schefer, U. (1992). Ruminant lactic acidosis in sheep and goats. *Veterinary Record* **130**, 343-349.
4. Das, N.K. (1979). Ruminant feed additive. United States Patent **4138498**, 1-14.
- 15 5. Dawson, K.A. & Allison, M.J. (1988). Digestive disorders and nutritional toxicity. In *The rumen microbial ecosystem*, pp. 445-459. Edited by P.N. Hobson. London: Elsevier Applied Science.
- 20 6. Gray, W. M. (1978). Microbial interactions in defined continuous culture systems effecting anaerobic cellulose degradation, PhD thesis: Clemson University.
- 25 7. Hession, A.O. & Kung, L., Jr. (1992). Altering rumen fermentation by microbial inoculation with lactate-utilising microorganisms. *J Anim Sci* **70**, 311. (Abstract)
8. Jannasch, H.W., 1977. Growth kinetics of aquatic bacteria. In: *Aquatic Microbiology*. Skinner, F .A. & Shewan, J .M. (Eds), Academic Press, London, 55-57.
- 30 9. Kung, L., Jr. & Hession, A.O. (1995). Preventing in vitro lactate accumulation in ruminal fermentations by inoculation with *Megasphaera elsdenii*. *J . Anim Sci* **73**, 250-256.
- 35 10. Krieg, R., 1981. Enrichment and isolation. In: *Manual of Methods for General Bacteriology*. Gerhardt, P. (Ed.), American Society for Microbiology, Washington, 112- 114.
- 40 11. Lederberg, J. & Lederberg, E.M. (1952). Replica plating and indirect selection of bacterial mutants. *J Bact* **63**, 399-406.
12. Leedle, J.A.Z., Greening, R.C. & Smolenski, W.J. (1991). Ruminant bacterium for preventing lactic acidosis. International Application No : PCT/US91/00857 1-41.
- 45 13. Mackie, R.I., Gilchrist, F.M.C., Robberts, A.M., Hannah, P.E. & Schwartz, H.M. (1978). Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *J agric Sci , Camb* **90**, 241-242.

14. Mackie, R.I., Gilchrist, F.M.C. & Heath, S. (1984). An in vivo study of ruminal micro-organisms influencing lactate turnover and its contribution to volatile fatty acid production. *J agric Sci, Camb* 103, 37-51.
- 5 15. Mackie, R.I. & Gilchrist, F.M.C. (1979). Changes in lactate-producing and lactate-utilising bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. *Appl Environ Microbiol* 38, 422-430.
- 10 16. Mackie, R.I. & Heath, S. (1979). Enumeration and isolation of lactate-utilising bacteria from the rumen of sheep. *Appl Environ Microbiol* 38, 416-421.
- 15 17. Marounnek, M., Fliegrova, K. & Bartos, S. (1989). Metabolism and some characteristics of ruminal strains of *Megasphaera elsdenii*. *Appl Environ Microbiol* 55, 1570-1573.
18. Olumeyan, D.B., Nagaraja, T.G., Miller, G.W., Frey, R.A. & Boyer, J.E. (1986). Rumen microbial changes in cattle fed diets with or without salinomycin. *Appl Environ Microbiol* 51, 340-345.
- 20 19. PIRT, S.J., 1975. Principles of Microbe and Cell Cultivation. Blackwell Scientific Publications, Oxford, 41-210.
20. Pryce, (1969). A modification of the Barker-Summerson method for the determination of lactic acid. *Analyst* 94, 1151.
- 25 21. Robinson, J.A., Smolenski, W.J., Greening, R.C., Ogilvie, M.L., Bell, R.L., Barsuhn, K. & Peters, J.P. (1992). Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii* 407A. *J Anim Sci* 70, 310. (Abstract)
- 30 22. Rogosa, M. (1984). Anaerobic Gram-negative cocci. In Bergey's manual of systematic bacteriology Volume 1, pp. 680-685. Edited by N.R. Krieg & J.G. Holt. Baltimore/London: Williams & Wilkins.
- 35 23. Russell, J.B. & Baldwin, R.L. (1978). Substrate preferences in rumen bacteria: evidence of catabolite regulatory mechanisms. *Appl Environ Microbiol* 36, 319-329.
- 40 24. Slyter, L.L. (1976). Influence of acidosis on rumen function. *J Anim Sci* 43, 910-929.
25. Stewart, C.S. & Bryant, M.P. (1988). The rumen bacteria. In The rumen microbial ecosystem, pp. 21-75. Edited by P.N. Hobson. London: Elsevier Applied Science.
- 45 26. Therion, J.J., Kistner, A. & Kornelius, J.H. (1982). Effect of pH on growth rates of rumen amylolytic and lactilytic bacteria. *Appl Environ Microbiol* 44, 428-434.

27. Van Gylswyk, N.O. (1990). Enumeration and presumptive identification of some functional groups of bacteria in the rumen of dairy cows fed grass silage-based diets. *FEMS Microbiol Ecol* **73**, 243-254.
- 5 28. Veldkamp, H. 1970. Enrichment cultures of prokaryotic organisms. In: *Methods in Microbiology*, Vol. 3A, Norris, J.R. & Ribbons, D.W. (Eds), Academic Press, London, 305-361.
- 10 29. Wilker, B.L., Paeye, L.M. & Baker, E. (1971). Bacterial culture for facilitating dietary adaptation of ruminants. Patent Office, London **1251483**, 1-13.
30. Wiryawan, K.G. & Brooker, J.D. (1995). Probiotic control of lactate accumulation in acutely grain-fed sheep. *Aust J Agric Res* **46**, 1555-1568.
- 15 **Cow experiments**
- 20 31. DAWSON, J.A. 1995. The use of yeast strain 8417 in manipulating ruminant high concentrate diets. Proc. 56th Minnesota Nutr. Conf. & Alltech Inc. Technical Symposium. September 18-20, Bloomington, MN.
32. DONOVAN, J. 1997. Subacute acidosis is costing no millions. *Hoard's Dairyman*, September 25 p. 666.
- 25 33. HALL, M.B. 1999. Management strategies against ruminal acidosis. Proc. Florida Ruminant Nutr. Symp. Univ. Florida, Gainesville, FL.
34. HUTJENS, M.F. 1995. Feeding applications for the high producing cow. Cornell Nutr. Conf. October 24-26. Dept. Anim. Sci., Cornell Univ., Ithaca, NY.
- 30 35. HUTJENS, M.F. 1999. How and when feed additives may or may not pay, *Hoard's Dairyman*, Sept 25, 1999.
- 35 36. KELLY, E.R. & LEAVER, J.D. 1990. Lameness in dairy cattle and the type of concentrates given. *Anim. Prod.* **51** : 221.
37. KUNG, L. 2000. Direct fed microbials for dairy cows. Proc. Florida Ruminant Nutr. Symp. Univ. Florida, Gainesville, FL.
- 40 38. KUNG, L. & HESSION, O.A. 1995. Altering rumen fermentation by microbial inoculation with lactate utilising micro-organisms. *J. Anim. Sci.* **73**:250.
39. MANSON, R.J. & LEAVER, J.D. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. *Anim. Prod.* **47** - 185.
- 45 40. McDANIEL, B.T. & WILK, J.C. 1989. Lameness in dairy cattle. Proc. Southwest Nutr. Mgmt. Conf., February 2-3, Dept. Anim. Sci., Univ. Arizona, Tucson, AZ.

41. NOCEK, J.E. 1995. Energy metabolism and rumen acidosis. Proc. Tri-state Dairy Nutr. Conf., May 23-24. Indiana Univ., Fort Wayne, IN.
- 5 42. NOCEK, H.E. 1997. Bovine acidosis. Implications on laminitis. *J. Dairy Sci* 80:1005.
43. NORDLUND, K.V. 1995. Questions and answers regarding rumenocentesis and the diagnosis of herd based sub-acute rumen acidosis. Proc. 4-State Applied Nutr. Conf., August 2-3, Univ. Wisconsin, Extension, Madison, WI.
- 10 44. NORDLUND, K.V., GARRET, E.F. & OETZEL, G.R. 1995. Herd based rumenocentesis: A clinical approach to the diagnosis of subacute rumen acidosis. The Compendium Food Animal, August 1995, p.48.
- 15 45. OETZEL, G.R. & SMITH, R.A. 2000. Clinical aspects of ruminal acidosis in dairy cattle. Proc. 33rd. Conf. American Assoc. Bov. Pract., Rapid City, SD. Sept 21 – 23.
- 20 46. OWENS, F.N., SECRIST, D., HILL, J. & GILL, D. 1996. A new look at acidosis. Proc. Southwest Nutr. Conf., Feb 1, Phoenix, AZ.
47. VOGEL, G.J. & PARROT, C. 1994. Mortality survey in feedyards: The incidence of death from digestive, respiratory and other causes in feedyards on the great plains. The Compendium February 1994: Food Animal, p. 227.
- 25 48. ROBINSON, J.A., SMOLENSKI, W.J., GREENING, R.C., OGILVIE, R.L., BELL, R.L., BARSUHN, K. & PETERS, J.P. 1992. Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii* 407A. *J. Anim Sci.* (Suppl. 1): 310 (Abstr)

30 Phylogenetics

49. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman, and K. Struhl. 1987. Current Protocols in Molecular Biology. Vol 1 and 2. John Wiley and Sons, New York,
- 35 50. Brosius, J., M.L. Palmer, P.J. Kennedy and H.F. Noller. 1978. Complete nucleotide sequence of the 16S ribosomal RNA gene from *E. coli*. Proc. Natl. Acad. Sci. 75:4801-4805.
- 40 51. Dorsch, M. and E. Stackebrandt. 1992. Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. *J. Microbiol. Methods.* 16:271-279.
- 45 52. Elsdén, S.R. and D. Lewis. 1953. The production of fatty acids by a gram-negative coccus. *Biochem. J.* 55:183-189.

53. Elsdén, S.R., B.E. Volcani, F.M.C. Gilchrist and D. Lewis. 1956. Properties of a fatty acid forming organism isolated from the rumen of sheep. J. Bacteriol. 72:681-689.
- 5 54. Engelmann, U. and N. Weis. 1985. *Megasphaera cerevisiae* sp. nov.: A new Gram-negative obligately anaerobic coccus isolated from spoiled beer. Syst. Appl. Microbiol. 6:287-290.
- 10 55. Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17:368-376.
- 15 56. Fox, G.E., J.D. Wisotzkey and P. Jurtshuk, Jr. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int. J. Syst. Bacteriol. 42:166-170.
57. *Genetics Computer Group. 1991. Program manual for the GCG package, version 7. Madison, Wisconsin, USA.
- 20 58. Gutierrez, J., R.E. Davis, I.L. Lindahl and E.J. Warwick. 1959. Bacterial changes in the rumen during the onset of feed-lot bloat of cattle and characteristics of *Peptostreptococcus elsdénii* n. sp. Appl. Microbiol. 7:16-22.
- 25 59. Haikara, A. 1992. The genera *Pectinatus* and *Megasphaera*, p.1993-2004. In A. Barlows, H. Trooper, M. Dworkin, W. Harder and K.-H. Schleifer (ed), The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, application, 2nd ed., vol ψψ. Springer Verlag, New York.
- 30 60. Hutson, R.A., D.E. Thompson, and M.D. Collins. 1993. Generic interrelationships of saccharolytic *Clostridium botulinum* types B, E and F and related clostridia as revealed by small-subunit rRNA gene sequences. FEMS Microbiol. Lett. 108:103-110.
- 35 61. Lane, D.J., B. Pace, G.J. Olsen, D.A. Stahl, M.L. Sogin, and N.R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA. 82:6955-6959.
62. Li, W. -H., and D. Graur. 1991. Fundamentals of molecular evolution. Sunderland, Massachusetts. Sinauer Associates Inc.
- 40 63. Maidak, B.L., Olsen, G.J., Larsen, N., Overbeek, R., McCaughey, M.J. and Woese, C.R. 1996. The ribosomal database project (RDP). Nucleic Acids Res 24:82-85.
- 45 64. Olsen, G.J., H. Matsuda, R. Hagstrom, and R. Overbeek. 1994. FastDNAmI - a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. CABIOS 10:41-48.

65. Rogosa, M. 1971. Transfer of *Peptostreptococcus elsdenii* Gutierrez et al. to a new genus, *Megasphaera* [*M. elsdenii* (Gutierrez et al.) comb. nov]. Int. J. Syst. Bacteriol. 21:187-189.
- 5 66. *Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning. A laboratory manual. Second edition (Eds. Ford, N., Nolan, C. and Ferguson, M.). Cold Spring Harbor, Laboratory Press.
- 10 67. Smith, S. 1992. Genetic Data Environment, version 2.0: Documentation. Harvard University & University of Illinois.
68. Stackebrandt, E., and W. Ludwig. 1994. The importance of choosing outgroup reference organisms in phylogenetic studies: the *Atopobium* case. Syst. Appl. Microbiol. 17:39-43.
- 15 69. *Stackebrandt, E., and F.A. Rainey. 1995. Partial and complete 16S rDNA sequences, their use in generation of 16S rDNA phylogenetic trees and their implications in molecular ecological studies. Molecular Microbial Ecology Manual 3.1.1:1-17. Kluwer Academic Publishers, Netherlands.
- 20 70. Stackebrandt, E., H. Pöhla, R. Kroppenstedt, H. Hippe, and C.R. Woese. 1985. 16S rRNA analyses of *Sporomusa*, *Selenomonas*, and *Megasphaera* : on the phylogenetic origin of Gram-positive Eubacteria. Arch. Microbiol. 143:270-276.
- 25 71. Stackebrandt, E., and O. Charfreitag. 1990. Partial 16S rRNA primary structure of five *Actinomyces* species: phylogenetic implications and development of an *Actinomyces israelii*-specific oligonucleotide probe. J. Gen. Microbiol. 136:37-43.
- 30 72. Stewart, C.S., and M.P. Bryant. 1988. The rumen bacteria. p. 21-75. In Hobson, P.N. (ed), The rumen microbial ecosystem., Elsevier Applied Science., London
- 35 73. Sugihara, P.T., V.L. Sutter, H.R. Attebery, K.S. Brichnell, and S.M. Finegold. 1974. Isolation of *Acidominococcus fermentans* and *Megasphaera elsdenii* from normal human feces. Appl. Microbiol. 27:274-275.
- 40 74. Utåker, J.B., L. Bakken, Q.Q. Jiang, and I.F. Nes. 1995. Phylogenetic analysis of seven new isolates of ammonia-oxidizing bacteria based on 16S rRNA gene sequences. Syst. Appl. Microbiol. 18:549-559.
- 45 75. Van Camp, G., Y. Van De Peer, S. Nicolai, J.-M. Neefs, P. Vandamme, and R. De Wachter. 1993. Structure of 16S and 23S ribosomal RNA genes in *Campylobacter* species: phylogenetic analysis of the genus *Campylobacter* and presence of internal transcribed spacers. Syst. Appl. Microbiol. 16:361-368.
- 50 76. Vandamme, P., B. Pot, M. Gillis, P. De Vos, K. Kersters, and J. Swings. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60:407-438.

77. van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2nd Edition. Comstock Publishing Associates, Ithaca.
- 5 78. Wiederhold, A.H. 1994. Isolation, selection and cultivation of lactic acid-utilising rumen bacteria for the treatment of chronic and acute acidosis. MSc. thesis. University of Natal, Pietermaritzburg.
- 10 79. Woese, C.R. 1987. Bacterial evolution. Microbiol. Rev. 51:221-271.